Bound Bone Water Density is a Surrogate Measurement of Organic Matrix Density

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Introduction: Proton NMR signal in bone arises from three pools: (1) T_2 > 1 ms, corresponding to free water within the Haversian and lacunocanalicular pore system, (2) T_2 ~ 400±100 µs, corresponding to motionally restricted water hydrogen-bonded to matrix collagen, and (3) T_2 << 100 µs, corresponding to highly immobilized ^{1}H nuclei in collagen [1]. These pools and their redistribution in response to increased porosity are diagrammed in a schematic T_2 spectrum (Fig. 1). Horch et al. [2] found the bound water pool in cortical bone, imaged using single adiabatic inversion recovery UTE imaging (SIR-UTE) for long-T_2 pore water-suppression, to correlate strongly with mechanical properties and short-T_2 bound water density by bi-exponential fitting of CPMG data. Cao et al. [3] also examined the correlation of long T_2-suppressed ^{1}H density to gravimetric matrix density in three porcine cortical specimens, and Ong et al. [4] found a negative correlation between porosity and bound water by ^{2}H IR. To provide a foundation for the use of ^{1}H SIR-zero TE (SIR-ZTE) imaging to measure matrix density (and, further, bone mineralization) in human cortical bone, we have examined a set of 15 specimens and compared bound water density with gravimetric matrix density and µCT porosity.

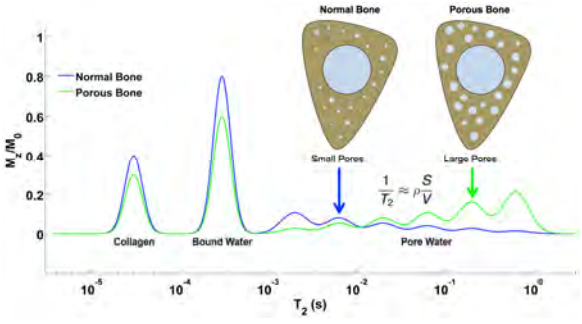

Fig. 1: Schematic T_2 spectrum of equilibrium ^{1}H signal in normal (blue) and porous (green) bones, showing the relative positions and sizes of collagen, collagen-bound water, and pore-resident water fractions. Porous bone has a smaller bound water fraction, and a larger pore water fraction with increased T_2.

Methods:

Specimens: 15 cylindrical samples of human cortical bone (8F, 27-97 y; 7M, 37-93 y) were cut from cadaveric human tibiae. The long axis of each cylindrical sample was perpendicular to the anatomic axis of the bone. Bone from donors with bone-demineralizing disorders was excluded.

MRI: Bones were imaged along with a reference phantom of 10 mM $MnCl_2$ in 90% D_2O/10% H_2O ([^{1}H] = 11.08 M) using an adiabatic inversion recovery-prepared version of a commercial ZTE pulse sequence on a 9.4T vertical-bore NMR spectrometer and micro-imaging scanner (Bruker, Billerica, MA). A 5-ms 5-kHz bandwidth adiabatic pulse inverts long-T_2 pore water magnetization while nulling short-T_2 bound water signal. After TI = 50 ms, readout gradients are ramped up, signal is excited with a 25.6-µs 60° RF pulse, and data are acquired at 100-kHz bandwidth 0.5-mm isotropic resolution. One half-projection is acquired following each inversion, with TR = 200 ms.

Analysis: The bone and reference phantom in each image were masked, and intensities were corrected for differences in relaxation times between bound bone water (T_1 = 480 ms, T_2* = 400 ms) and the reference phantom (T_1 = 13 ms, T_2* = 530 µs). Transverse relaxation during adiabatic and excitation RF pulses was simulated and incorporated, and signal intensities were converted to ^{1}H concentrations.

µCT: Bones were scanned on a Scanco µCT35 scanner (Scanco, Brüttisellen, Switzerland) at 18.5-µm isotropic resolution. Bones were masked by active snakes in ITK-SNAP [5], and pores were segmented by thresholding, yielding porosity as pore volume / total volume.

Gravimetry: The fully hydrated samples were weighed, dried at 105° C for 110 hr to remove all bound and pore water, re-weighed, ashed at 600° C for 30 hr to burn off all organic matrix, and weighed again. Organic matrix density was quantified as the difference between dry and ash masses divided by total volume measured by µCT.

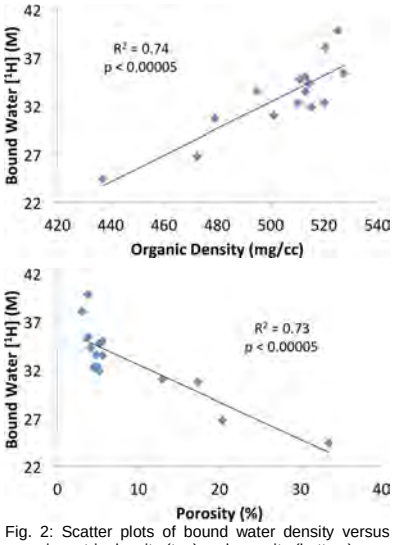

Fig. 2: Scatter plots of bound water density versus organic matrix density (top) and porosity (bottom).

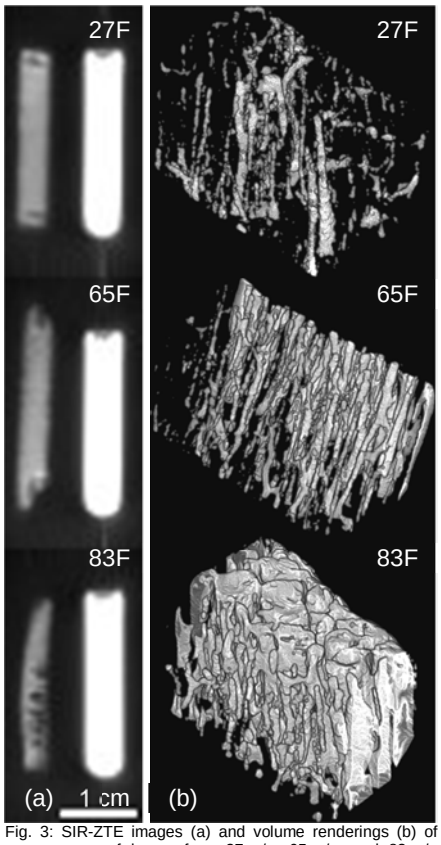

Fig. 3: SIR-ZTE images (a) and volume renderings (b) of pore spaces of bones from 27 y/o, 65 y/o, and 83 y/o females, demonstrating the range of porosities (3.7%, 5.6%, and 33.5%, respectively). In panel (a), bones are on the left and the reference phantom is on the right.

Results: Bound water ^{1}H concentration by SIR-ZTE is correlated positively with organic density (R^2 = 0.74) and negatively with µCT porosity (R^2 = 0.73) (Fig. 2). Bound water is also correlated with gravimetric mineral density (R^2 = 0.72), which is, in turn, also strongly correlated with matrix density (R^2 = 0.91). Organic density and porosity are also strongly associated (R^2 = 0.91). Pore-space volume renderings of bone differing substantially in porosity are shown in Fig. 3. Pore volume fraction and pore water concentration increase proportionally, and pore water T_2 also increases as individual pores enlarge and the contribution of surface relaxation to T_2 is diminished.

Discussion and Conclusions: The strong correlation of long T_2-suppressed ^{1}H density in cortical bone to matrix density and porosity further supports the potential of this method as a surrogate for matrix density. The association between gravimetric matrix and mineral densities shows that mineralization, in the absence of demineralizing disorders, is constant over a wide range of ages. As ^{1}H SIR-UTE has already been applied in vivo [2], this entirely non-invasive SIR-ZTE method may provide detailed insight into bone chemistry and composition in human subjects.

References: [1] Horch RA. MRM 2010;64:680-7. [2] Horch RA. MRM 2012;68(6):1774-84. [3] Cao H. MRM 2008;60(6):1433-43. [4] Ong HH. JBMR 2012;27(12):2573-81. [5] Yushkevich PA. Neuroimage 2006;31(3):1116-28.

Acknowledgements: NIH R01 AR50068, F31 AG042289, UL1TR000003, and Institution's ITMAT.